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Identity and neotypification of *Craterellus cinereus* and description of *Cantharellus atrofuscus* sp. nov.

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Abstract — The study of authentic specimens of *Cantharellus cinereus*, present in the Leiden Herbarium (L), has allowed us to ascertain the identity of this species, which has no clamp connections. There exists a species close to *Craterellus* (*Cantharellus*) *cinereus* distinguished by important macro- and microscopic features, including an unperforated basidioma and presence of clamp connections. This species is here proposed as new to science with the name *Cantharellus atrofuscus*. A description and photos of habit and microscopic characters are provided.

Keywords — *Basidiomycota*, *Agaricomycetes*, *Cantharellales*, taxonomy, Italy

Introduction

The problem of the identity of *Cantharellus cinereus,* i.e. *Merulius cinereus*, also known as *Craterellus cinereus*, has long been an enigma for the present authors because, judging from the literature and our personal collections, two different species have been described and illustrated under this name, with one species characterized by clamped hyphae (Corner 1966, Jülich 1989, Ellis & Ellis 1990, Romagnesi 1995) and the other by clampless hyphae (Kühner & Romagnesi 1953, Donk 1969, Marchand 1973, Bigelow 1978, Breitenbach & Kränzlin 1986, Persson & Mossberg 1994, 1998; Knudsen et al. 1997, Pegler et al. 1997, Watling & Gregory 1998).

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Donk (1969) was the first author to recognize the different interpretations of the taxon "*cinereus*". In fact, when he discovered that Persoon's authentic material preserved in Leiden consisted of basidiomata with clampless hyphae, he stated that the clamp-bearing species described by Corner as "*Cantharellus cinereus*" must represent another taxon. Similar observations were also made by Bigelow (1978), who, after studying some American clampless collections labeled as "*Craterellus cinereus* (Fries) Quélet", noted that two different interpretations existed in the mycological literature: a clampless taxon described by Kühner & Romagnesi (1953) and ascribed to *Craterellus* Pers. and a clamped one referred by Corner (1966) to *Cantharellus* Juss. Bigelow (1978), who affirmed that it was impossible to distinguish the two taxa macroscopically, observed that *Cantharellus cinereus* s. Corner (1966) seemed to produce larger spores than the clampless species. For that reason he concluded that it was not possible to determine the real distribution of these two taxa, although he regarded *C. cinereus* s. Kühner & Romagnesi (1953) as more frequent and widespread than *C. cinereus* s. Corner (1966), at least judging from the spore sizes of collections cited in the literature.

In his cantharelloid monograph, Corner (1966) cited many descriptions and illustrations to support his concept of "*Cantharellus cinereus*", emphasizing that he had examined collection 1777 Fungi Exsiccati Suecici (Lundell & Nannfeldt 1949) but failing to specify whether that material was clamped or not. Nevertheless he regarded his *Cantharellus cinereus* as "a true Cantharellus, both in the construction of the gill-folds and secondarily in the hollow nature of the pileus and stem."

The description of *Merulius cinereus* given by Persoon (1801) is quite short but the data are definitely important; the Dutch author described *Merulius cinereus* as "caespitosus, pileo subinfundibuliformi squamuloso nigrescente, plicis cinereis nitidis, stipite cavo nigrescente" growing "in faginetis, locis apertis" and different from *Merulius cornucopioides* in possessing well formed gill-like folds. In this description "*Helvella hydrolips* Bull. Hist. d. champ. 1. p. 212. t. 565. f. 1" was recorded as a variety of *M. cinereus*.

Our only opportunity to ascertain the real identity of Persoon's agaric was to examine the authentic material conserved in Leiden (L). For this reason, M. Carbone visited that herbarium and examined all the collections labeled "*Merulius cinereus*".

Below we provide the results of this revision; we clarify the concept of *Craterellus cinereus* and describe the species exhibiting clamp connections as new to science.

Materials and methods

The description of macroscopical features of *Cantharellus atrofuscus* sp. nov. (vide infra) is based on fresh material. Micro-features of *C. atrofuscus* and Persoon's material are

based on dried specimens, rehydrated in L4 or KOH 5% solutions, and then mounted in Red Congo to observe the hymenium and pileipellis, and in water for spore dimensions. L4 is a solution composed by water, KOH, NaCl, Invadin Ciba, phenol, and glycerine.

The number of the measured spores has involved many specimens and many collections, in order to calculate a reliable range of spore dimensions. We chose all the spores present in the visual field to satisfy the random principle. Basidiospore measurements do not include the apiculus. Spore dimensions are described as Length (mean – mean square deviation)–(mean + mean square deviation) × Width (mean – mean square deviation)–(mean + mean square deviation). The following abbreviations are used: Q = the spore quotient (length/width ratio); Qm = the average spore quotient; Vm = the average spore volume.

Author citations are according to the IPNI Authors website and the Index Fungorum Authors of Fungal Names website.

Cantharellus cinereus

In the National Herbarium Nederland of Leiden (L) there are two folders labeled "*Merulius cinereus* Pers." The first includes four collections: 1) L0111397 nr. 910.255-41 (labeled "type"); 2) L0111398 nr. 910.255-27; 3) L0111399 nr. 910.255-61; 4) L0111400 nr. 910.255-47. The second folder includes a single collection, L0111401 nr. 910.255-19.

First folder

Handwriting on the herbarium sheets shows that collections 1–3 were already revised by Donk, who reported his observations in Donk (1969). M. Carbone's reexamination results follow:

L0111397 (Fig. 1) — This collection is labeled as "type", although no official designation has been proposed to our knowledge (see also Donk, 1969). Here we designate L0111397 as "neotype" because evidence is lacking that it was in Persoon's hands when the name was published (Persoon 1794). Epitypification is excluded because the original diagnosis lacked drawings and/or reference to other plates.

Micromorphological features are reported in the following section "Neotypification of *Cantharellus cinereus*".

L0111398 — Basidia 4-spored, 55–65 µm, lacking basal clamps. Subhymenium hyphae without clamps. Spores 9–10 × 5–6 µm. Pileipellis made up of cylindrical hyphae to 10 µm wide, sometimes anastomosed, with occasional secondary septa. Clamp connections lacking in all tissues.

Persoon labeled this collection "*Merulius cinereus*" and also wrote on the herbarium sheet that *Helvella hydrolips* Bull. 1790 was the same taxon.

There's also a short note by Bas, date XII.1968: "Hymenium on fold near margin of cap. Basidia without clamps!"

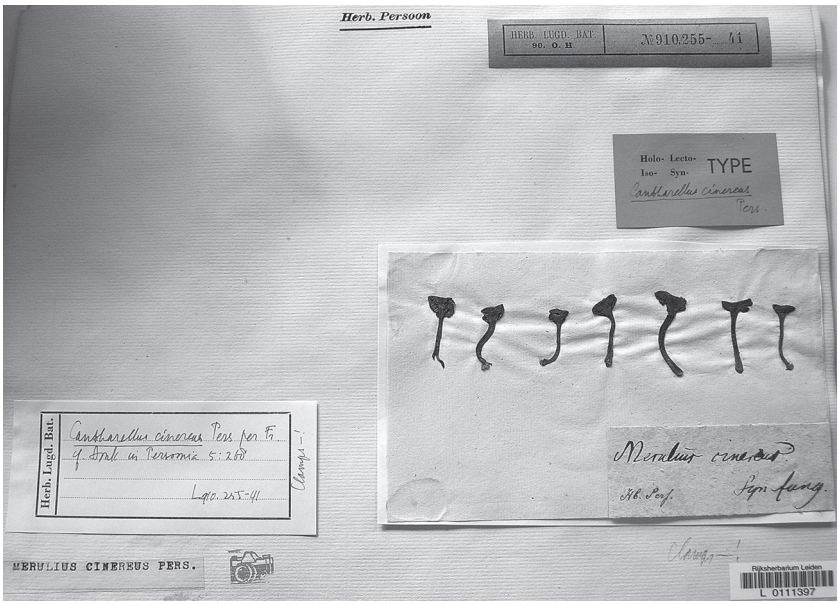

Figure 1. *Craterellus cinereus* (neotype).

L0111399 — Basidia 4-spored, on average 55 µm long, lacking basal clamps. Subhymenium hyphae without clamps. Spores 9–11 × 5.5–6.5 µm. Pileipellis made up of cylindrical hyphae; secondary septa present; clamp connections lacking throughout.

In the herbarium sheet are some notes in Persoon's handwriting: 1) the collection represents *Merulius cinereus* Syn. Fung.; 2) specimens sent to Persoon by "Raddi", probably Giuseppe Raddi (1770–1829), a Florentine mycologist known for describing *Boletus rubropunctatus*, an earlier synonym of *Boletus bellinii* Inzenga 1879; 3) probably in Persoon's handwriting, "espèce très rare chez nous".

L0111400 — Basidia 4-spored, 50–55 µm, lacking basal clamps. Subhymenium hyphae without clamps. Spores 9.5–10 × 6 µm. Pileipellis made up of cylindrical hyphae to 10 µm wide, secondary septa present (but less numerous than in the other collections). Clamp connections lacking in all tissues.

From the herbarium sheet it seems that this collection, labeled "*Cantharellus cinereus*", was not identified by Persoon but by Leveillé. In fact the name "*Cantharellus cinereus*" is followed by a short note: "Leveillé scrips.". However, also in this case, the microfeatures support it as representing Persoon's "*cinereus*".

Second Folder

L0111401 — Basidia 2- and 4-spored, 70 µm, lacking basal clamps, as in the subhymenium. Spores 9.5–11 × 7–8 µm. Pileipellis made up of cylindrical hyphae to 10 µm wide, secondary septa not seen.

Donk, who studied this collection, ascribed it to "*Pseudocraterellus.*" Based on two-spored basidia and different spore morphology, M. Carbone believes the basidiomata to represent *Craterellus undulatus* (Pers.) Redeuilh 2004.

Neotypification of *Cantharellus cinereus*

Craterellus cinereus (Pers. : Fr.) Donk, Meded. Ned. Mycol. Ver. 22 : 67. 1933, nom. illeg. but see the proposal of conservation by Olariaga et al. (2009).

Figs. 1, 2

(non *Craterellus cinereus* Pers., Mycol. Europ. 2: 6, 1825).

≡ *Cantharellus cinereus* Pers. : Fr., Neues Mag. Bot. 1: 106. 1794.

≡ *Merulius cinereus* (Pers. : Fr.) Pers., Icon. Descr. Fung. 1: 10, tab. 3, fig. 3. 1798.

≡ *Xerocarpus cinereus* (Pers. : Fr.) P. Karst., Rev. Mycol. (Toulouse) 3(9): 22. 1881.

≡ *Pseudocraterellus cinereus* (Pers. : Fr.) Kalamees, Tartu Riik. Ülik. Toim. 136: 90. 1963.

Neotypus hic designatus: "L0111397 = 910.255-41, *Merulius cinereus* Pers." (L).

We designate this collection as neotype of *Cantharellus cinereus* Pers. 1794, following and agreeing with Donk (1969), who was the first to select it as "type" although not formally and not in a printed publication (see the packet in L).

The seven small basidiomata are in good state of conservation and are mature enough for microscopical studies (Fig. 1).

Donk's reference (1969) to number 910.255-14 was surely a misprint. His description perfectly fits 910.255-41.

Macroscopic features

As originally indicated by Persoon (1794, 1801, 1825).

Microscopic features

Basidia 4(–5)-spored, 55–60 µm long, without basal clamps. Subhymenium hyphae without clamps. Spores 9–10 × 5–6 µm (Fig. 2D). Pileipellis made up of cylindrical hyphae up to 10 µm wide, secondary septa present (Fig. 2B). Clamp connections lacking in all tissues.

After revision of Persoon's material, including the collection here designated as neotype, it is clear that for "*Cantharellus cinereus*", i.e. *Craterellus cinereus*, Persoon meant an unclamped species with spores never exceeding 11 µm long.

Consequently, the original concept of the taxon "*cinereus*" conforms to the one adopted by most authors who studied the species giving detailed descriptions also from a micromorphological point of view.

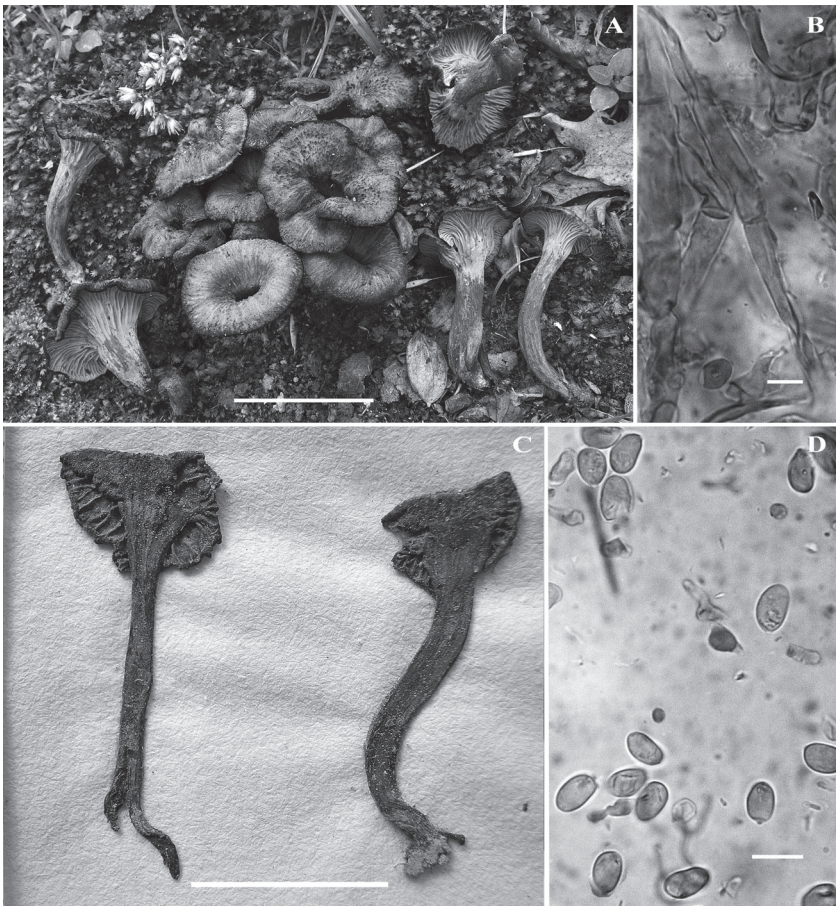

Figure 2. *Craterellus cinereus*.
A. Fresh basidiomes in situ. B–D. Neotype. B. Pileipellis. C. Basidiomes. D. Spores.
Bars: A = 3 cm; B, D = 10 µm; C = 1 cm.

Proposal of a new species

For the reasons given above, the taxon exhibiting clamp connections and described as "*Cantharellus cinereus*" by Corner (1966), Jülich (1989), Ellis & Ellis (1990), and Romagnesi (1995) does represent a different species without an available and eligible valid name, so the introduction of a new species is necessary. We consider this species to represent not a *Craterellus* but a true *Cantharellus* characterized by a non-perforate pileus, well developed, cantharelloid gill-folds (Corner 1966), and a fibrous, solid stipe.

Cantharellus atrofuscus Contu, Vizzini, M. Carbone & Setti, **sp. nov.** Fig. 3

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Misapplied: *"Cantharellus cinereus"* sensu Corner (1966), Jülich (1989), Ellis & Ellis (1990), Romagnesi (1995); non *Cantharellus cinereus* Pers. 1794.

Basidiomata usque ad 70 mm alta, caespitosa, simplicia, haud pluripileata. Pileus 10–80 mm latus, modice carnosus, depressus vel infundibuliformis sed haud perforatus, reflexus ad expansum, minute fibrillosus, niger vel atrofuscus deinde ochraceo-griseus. Hymenium venosum, ex plicis pseudolamellaribus griseis, saepe intervenosis vel anastomosis, obtusis efformatum. Stipes 30–70 × 3–10 mm, cylindraceus, haud compressus, versus basim albidus vel flavidus, aliunde concolor cum pileo, levis, solidus, subplenus vel fibroso-plenus. Caro modice conspicua, pallide griseo-brunnea, immutabilis. Odor ut in Muscari racemosa; sapor mitis. Sporae 9.4–10.5 × 8.3–9.4 µm, hyalinae, regulariter ellipsoideae, late ellipsoideae, obtusae, pluriguttulatae, parietibus leviter incrassatis. Basidia 57–83 × 9.8–13 µm, tetraspora, clavata, fibulata. Pilei cutis ex hyphis cylindraceis ad apicem angustatis, 4.5–12.5 µm, latis constituta, pigmento intraparietali et vacuolari. Septa secondaria rara vel nulla. Fibulae numerosae.

*Hab.: ad terram, in silvis. Autumno-hieme. Typus: Italia, Sardegna, prov. Sassari, Tempio Pausania, loc. Baldo, in nemore frondoso acido cum Quercu subere, 26.10.2002, leg. G. Consiglio (Erbario AMB, n. 1, **holotypus**).*

Pileus 10–80 mm, thin-fleshed, largely depressed but with solid flesh, not appreciably perforate in old specimens, very undulate, deep anthracite black, somewhat discolored to pale grey-brownish in age; surface typically with long and thick radial fibrils, slightly darker than the background color, margin often lobed (Fig. 3A). Black with KOH. Hymenium with well formed and relatively thick gill-like folds, forked and interveined, slightly decurrent to decurrent, ash grey, gill-fold edges blunt. Stem 30–70 × 3–10 mm, fibrous and solid for a long time, sub-hollow only in some overmature specimens, mainly clavate, dry, with long and thick longitudinal fibrils, concolorous with pileus, white to yellow toward the base. Context quite firm, pale brownish grey, unchanging. Smell fruity [reminiscent of *Muscari racemosum* (L.) Mill.], quite pleasant. Taste mild. Spore-print white.

Spores 9.4–10.5 × 8.3–9.4 µm, Q = 1.08–1.18, Qm = 1.13, Vm = 412 µm^3, subglobulose to widely ellipsoid, with non-refractive granular contents, inamyloid, smooth (Fig. 3D). Basidia 57–83 × 9.8–13 µm, (2–)4-spored, narrowly clavate; sterigmata up to 8 µm long, slightly curved inward (Fig. 3C). Hymenophoral trama irregular, made up of hyphae to 20 µm wide, hyalinae in L4 mounts, yellow in Melzer reagent. Pileipellis a cutis of hyphae variously twisted, up to 12.5 µm wide, not gelatinized, yellow in Melzer reagent, smooth, cylindrical; terminal elements smooth, to 6 µm wide, with more or less cream-colored intracellular pigment, some with a very thin incrusting pigment (Fig. 3B). Tramal hyphae cilindrical, up to 10 µm wide. Stipe hyphae cylindrical, in medulla 8 µm diam., in cortex 5 µm diam, smooth, yellow in Melzer, terminal elements smooth. Clamp connections present in all tissues.

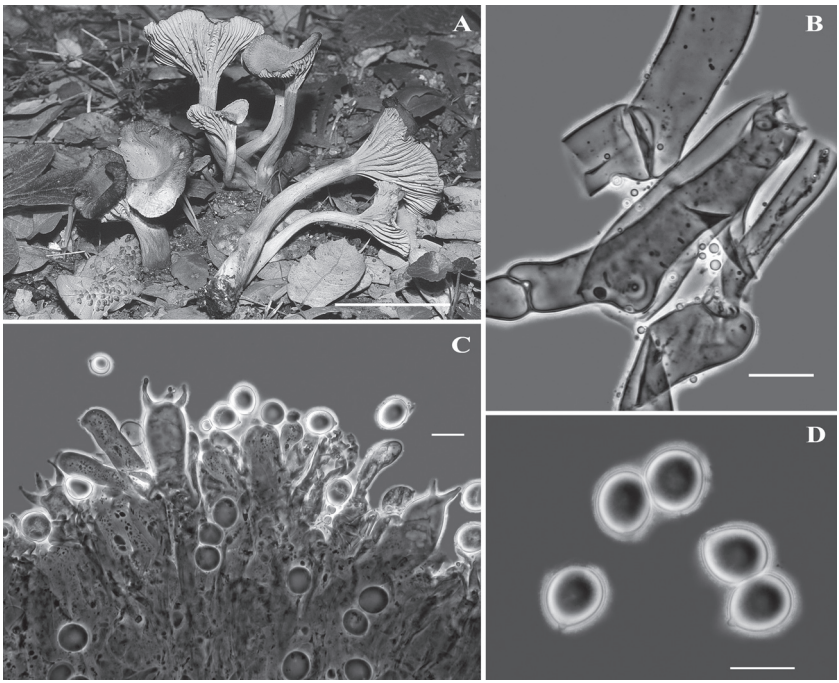

Figure 3. *Cantharellus atrofuscus* (holotype).
A. Fresh basidiomes in situ. B. Pileipellis. C. Hymenium. D. Spores.
Bars: A = 5 cm; B, C, D = 10 µm.

Habitat: cespitose, both in hardwood and conifer or also mixed forests. In autumn and winter.

Distribution: so far surely known from France and Italy, but probably more widespread, due to the confusion with *C. cinereus*.

Additional material studied. Italy: Sardinia, prov. Sassari, Monte Limbara, loc. S'Ampulla, under *Quercus ilex* L., 25.10.2002, leg. M. Contu (CAG); ibidem, prov. Nuoro, Villagrande, loc. Bosco di S. Barbara di Villagrande, 27.10.1999, leg. F. Padovan e M. Floriani (studied but not conserved).

Discussion

Based on molecular studies by Feibelman et al. (1997) and Dahlman et al. (2000), delimitation of the cantharelloid genera has undergone many changes. Those studies established that *Cantharellus* s. str. must be limited to the *Cantharellus cibarius* complex (for an actual definition of the genus cfr. Eyssartier & Buyck 2000), while *Pseudocraterellus* Corner must be included in *Craterellus* (Feibelman et al. 1997, Dahlman et al. 2000, Moncalvo et al.

2006). For that reason *Craterellus* should include species with funnel-shaped basidiomes, with (completely or partly) hollow stipes and with or without clamps (sect. *Leptocantharellus* Peck included). Placement of our new species within *Cantharellus* is supported by the presence of a solid stipe and a non-funnel-shaped pileus as in the *Cantharellus cibarius* complex.

As shown above, *Cantharellus atrofuscus* has often been confused with *Craterellus cinereus*, from which it differs in the following features (Table 1): 1) pileus depressed but almost never perforate in mature specimens; 2) gill-like folds better defined, thicker, and with a sharp edge; 3) smell strong, fruity and not weak or aromatic; 4) larger and more rounded spores; 5) abundant clamp connections on basidiome hyphae in which secondary septa are rare or absent, whilst they are abundant in the hyphae of *C. cinereus*.

Macroscopical differences are not always easy to ascertain in the field, so microscopical studies are required to make a correct identification. The stipe morphology could help differentiate fresh, well formed basidiomata of both species: in *Craterellus cinereus* it is always hollow from the young stages whilst it is always fibrous and solid to sub-hollow in *Cantharellus atrofuscus*.

While *C. cinereus* could be confused with many species (Singer 1963, Corner 1966, Petersen 1969, Bigelow 1978, Grgurinovic 1997), on the contrary *Cantharellus atrofuscus*, once surely identified, can be confused with very few. The similar *Cantharellus congolensis* Beeli 1928 differs in more crowded gill-like folds, flesh that discolors from red to black, lack of odor, smaller (5.3–7.3 × 3.9–4.7 μm) spores, and the pseudoparenchymatic structure of subhymenium and stipe trama (Heinemann 1958).

Craterellus cornucopioides (L.) Pers. 1825 (*C. fallax* A.H. Sm. 1968 included, see Dahlman et al. 2000) differs in producing a deeply funnel-shaped, hollow basidiome, a quite smooth hymenophore, hyphae without clamps, predominantly 2-spored basidia, and bigger (10–14 × 8–11 μm) spores (e.g. Knudsen et al. 1997).

Craterellus melanoxeros (Desm.) Pérez-De-Greg. 2000 is easily distinguished by the long-lasting yellow coloration, white flesh, non-fruity smell, narrower spores, and different pileus cuticle structure (Neville & Alpago-Novello 1998).

Among North American taxa, *Craterellus venosus* R.H. Petersen (Petersen 1975) differs in having a perforate basidiome, hyphae without clamp connections, strictly 6-spored basidia, and narrower (8.1–10 × 4.8–5.6 μm) spores, while *Craterellus caeruleofuscus* A.H. Sm. (Smith 1968, Bigelow 1978) is characterized by a perforate basidiome, a glabrous pileus, a venose hymenophore, hyphae without clamp connections, 6-(rarely 2-)spored basidia, and smaller (7–8.5(–9) × 5–6(–6.5) μm) spores.

Table 1. Main distinguishing characters between *Craterellus cinereus* and *Cantharellus atrofuscus*

Species	Basidiomes	Gill-like folds	Basidiospores	Hyphae
C. cinereus	Pileus and stipe perforated ("cornucopioid")	Sharp edged	9–10 × 5–6 µm, narrowly ellipsoid	Clamp connections absent; secondary septa abundant
C. atrofuscus	Pileus and stipe not perforated	Blunt edged	9.4–10.5 × 8.3–9.4 µm, subglobose to broadly ellipsoid	Clamp connections present; secondary septa rare

Acknowledgements

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